

Amendments to the Specification

Please replace the paragraph beginning on p. 23, line 25 with the following amended paragraph (underlining of journal volumes in original):

By "essential HSV genes", it is intended that the one or more vectors include all genes that encode polypeptides that are necessary for replication of the amplicon vector and structural assembly of the amplicon particles. Thus, in the absence of such genes, the amplicon vector is not properly replicated and packaged within a capsid to form an amplicon particle capable of adsorption. Such "essential HSV genes" have previously been reported in review articles by Roizman (*Proc. Natl. Acad. Sci. USA* 11:307-1 13, 1996; *Acta Viroloica* 43:75-80, 1999) and include, *U₁1*, *U₁5*, *U₁6*, *U₁7*, *U₁8*, *U₁9*, *U₁14*, *U₁15*, *U₁17*, *U₁18*, *U₁19*, *U₁22*, *U₁25*, *U₁26*, *U₁26.5*, *U₁27*, *U₁28*, *U₁29*, *U₁30*, *U₁31*, *U₁32*, *U₁33*, *U₁34*, *U₁35*, *U₁36*, *U₁37*, *U₁38*, *U₁42*, *U₁48*, *U₁49*, *U₁49.5*, *U₁52*, *a27*, *a4*, *U₅7*. Another source for identifying such essential genes is available at the Internet site operated by the Los Alamos National Laboratory, Bioscience Division, which reports the entire HSV-1 genome and includes a table identifying the essential HSV-1 genes.

Please replace the paragraph beginning on p. 34, line 9 with the following amended paragraph (underlining of journal volumes in original):

The previously described HSV λ c amplicon contains the coding sequence for *E. coli* β -galactosidase under the transcriptional control of the HSV immediate-early 4/5 gene promoter (Geller and Breakefield, *Science* 241:1667-9, 1988). The 126-bp sequence encoding β 1-42 was PCR-amplified using sequence-specific primers that contained Bam HI and Hind III restriction sites and cloned into the HSVPrPUC amplicon vector (Geller and Breakefield, *Science* 241 : 1667-9, 1988) to create HSV λ β . The β 1-42 sense primer was 5' - CCCGAAGCTTACCATGGATGCAGAAATCCGACATGACTCAGG-3' (SEQ ID NO:1) and the β 1-42 sense primer was 5'-CCCGAAGCTTACCATGGATGCAGAAATCCGACATGACT-CAGG-3' (SEQ ID NO:2). HSV λ β /TtxFC was constructed by PCR amplifying the 1356-bp tetanus toxin fragment C segment (TtxFC) using gene-specific primers that contained *Bam*HI and *Sac*I restriction sites and the resultant product was cloned into the HSV λ β vaccine vector. The TtxFC sense primer was

5'- GCGGGATCCAAAAATCTGGATTGTTGGGTTGATAAT-3' (SEQ ID NO:3) and the TtxFC antisense primer was 5'-CGACTGAGCTCTTAATCA-TTTGTCCATCCTTCATCTGT-3' (SEQ ID NO:4). The newly designed vectors were sequenced to confirm identity, and in the case of HSV α /TtxFC, to ensure the maintenance of translational reading frame between A β l-42 and TtxFC coding sequences. Amplicon stocks were prepared using a modified helper virus-free packaging method that has been described previously (Bowers et al, Gene. Ther. 8:111-120, 2001). Briefly, on the day before transfection, 2×10^6 BHK cells were seeded on a 60-mm culture dish and incubated overnight at 37°C. For cosmid-based packaging: the day of transfection, 250 μ l Opti-MEM (Gibco-BRL, Bethesda, MD, USA), 0.4 μ g of each of the five cosmid DNAs (kindly provided by Dr A Geller, Children's Hospital, Boston, MA, USA) and 0.5 μ g amplicon vector DNA with or without varying amounts of pBSKS(vhs) plasmid DNA were combined in a sterile polypropylene tube. For BAC-based packaging: 250 μ l Opti-MEM, 3.5 μ g of pBAC-V2 DNA (kindly provided by Dr C Strathdee, JP Roberts Institute, London, ON, Canada) and 0.5 μ g amplicon vector DNA with or without varying amounts of pBSKS(vhs) plasmid DNA were combined in a sterile polypropylene tube. The protocol for both cosmid- and BAC-based packaging was identical from the following step forward. Ten microliters of Lipofectamine Plus Reagent (Gibco-BRL) were added over a 30-s period to the DNA mix and allowed to incubate at RT for 20 min. In a separate tube, 15 μ l Lipofectamine (Gibco-BRL) were mixed with 250 μ l Opti-MEM. Following the 20-min incubation, the contents of the two tubes were combined over a 1-min period, and incubated for an additional 20 min at RT. During the second incubation, the medium in the seeded 60-mm dish was removed and replaced with 2 ml Opti-MEM. The transfection mix was added to the flask and allowed to incubate at 37°C for 5 h. The transfection mix was then diluted with an equal volume of DMEM plus 20% FBS, 2% penicillin/streptomycin, and 2 mM hexamethylene bis-acetamide (HMBA), and incubated overnight at 34°C. The following day, medium was removed and replaced with DMEM plus 10% FBS, 1% penicillin/streptomycin, and 2 mM HMBA. The packaging flask was incubated an additional 3 days and virus harvested and stored at -80°C until purification. Viral preparations were subsequently thawed, sonicated, and clarified by centrifugation (3000 g, 20 min). Vector titers were determined using expression- and transduction-based methodologies (Bowers et al, Mol. Ther. 1(3):294-299, 2000).